IN THE CLAIMS

Please cancel claims 64, 81, 88 and 89 and amend claims 63, 65, 72, 73, 79, 81, 82, 87 and 90.

Claims 1-62 (cancelled)

63. (currently amended) A method for generating ~~a specified amino acid, other than methionine~~ a cysteine or selenocysteine at the N-terminus of a target protein, comprising:

expressing in a host cell, a nucleic acid encoding a fusion protein having an intein coding sequence adjacent to a coding sequence for the cysteine or selenocysteine~~the specified amino acid other than methionine~~ in the target protein; and

cleaving the intein from the target protein so as to generate the cysteine or selenocysteine~~. specified~~ at the ~~N-terminal amino acid~~ N-terminus.

64. (cancelled)

65. (currently amended) A method for ligating a ~~plurality of~~ target proteins, the method comprising the steps of:

(a) expressing from a first plasmid in a first host cell, a first fusion protein comprising a first target protein having a C-terminus fused to an intein or modification thereof;

(b) expressing from a second plasmid in the first host cell or a second host cell, a second fusion protein comprising the second target protein having an N-terminus fused to an intein or modification thereof;

(c) obtaining an extracellular preparation of the first fusion protein and an extracellular preparation of the second fusion protein;

(d) adding a thiol reagent to the extracellular preparation of the first fusion protein whereby the first intein is cleaved so as to form a C-terminal thioester on the first target protein;

(e) cleaving the second intein or modification thereof from the second target protein in the extracellular preparation of the second fusion protein and forming an N-terminal cysteine or selenocysteine on the second target protein; and

(f) permitting ligation of the first target protein <u>with the c-terminal thioester</u> ~~of step (d)~~ with the second target protein of step (e).

66. (previously added) The method of claim 65, wherein the first intein is an Mth RIR1 intein (SEQ ID NO:24), wherein the Mth RIR1 is modified.

67. (previously added) The method of claim 65, wherein the second intein is an Mth RIR1 intein (SEQ ID NO:24), wherein the Mth RIR1 intein is modified.

68. (previously added) The method of claim 66 or 67, wherein the modification of the Mth RIR1 intein comprises Ala instead of Asn^{134} at the C-terminus, or Ala or Ser instead of Cys^{1} at the N-terminus.

69. (previously added) The method of claim 65, wherein the second target protein of step (e) is cleaved from the second intein in the presence of a thiol reagent or by modulating any of temperature, pH, salt, chaotropic agents or combinations thereof.

70. (previously added) The method of claim 65, wherein step (c) further comprises: purifying from the extracellular preparation, the first or second fusion protein.

71. (previously added) The method of claim 70, wherein the step of purifying the fusion protein further comprises binding to a chitin resin column.

72. (currently amended) The method of claim 65, wherein the first and second plasmids are capable of expression in ~~at least one cell type~~ a host cell selected from the group consisting of a bacterial, a yeast, a plant, an insect and a mammalian host cell ~~type~~.

73. (currently amended) A method for ligating a first and a second target protein, comprising:

~~(a) combining in a mixture~~

~~(i) a first target protein having a C-terminus, wherein the C-terminus comprises a thioester formed by cleavage of a first intein or modification thereof from a first fusion protein, the first fusion protein comprising the first intein or modification thereof positioned at the C-terminus of the first target protein; and~~

~~(ii) a second target protein having an N-terminus, wherein the N-terminus is a cysteine or selenocysteine amino acid, the N-terminus cysteine or selenocysteine resulting from induced cleavage of a second intein or modification thereof in a second fusion protein, the second fusion protein comprising the second intein or modification thereof positioned at the N-terminus of the second target protein;~~

(a) inducing cleavage of a first intein or modification thereof from a fusion protein comprising the intein and a first target protein, to form an N-terminus cysteine or selenocysteine amino acid on the target protein;

(b) combining in a mixture the first target protein of (a) with a second target protein having a C-terminus thioester; and

(c) ligating the first and second target proteins.

74. (currently amended) A method for cyclization of a target protein, the method comprising the steps of:

(a) expressing from a plasmid in a host cell, a fusion protein comprising a target protein having a C-terminus and an N-terminus wherein a first intein or modification thereof is fused at the C-terminus, and a second intein or modification thereof is fused at the N-terminus adjacent to a cysteine or selenocysteine amino acid on the target protein;

(b) obtaining an extracellular preparation of the expressed fusion protein;

(c) cleaving the fusion protein to remove the first and second inteins and obtaining the target protein having a C-terminal thioester and an N-terminal cysteine or selenocysteine; and

(d) permitting intramolecular ligation of the N-terminus to the C-terminus of the target protein to form a cyclized protein.

75. (previously added) The method of claim 74, wherein the intein is an Mth RIR1 intein (SEQ ID NO:24), and the Mth RIR1 is modified wherein

the modification comprises at the C-terminus, Ala instead of Asn^{134} or at the N-terminus, Ala or Ser instead of Cys^{1}.

76. (previously added) The method of claim 74, wherein the N-terminal cysteine or selenocysteine is formed by cleavage of the intein or modification thereof from the target protein by modulating any of temperature, pH, salt, chaotropic agents or combinations thereof and the C-terminal thioester on the target protein is formed by adding a thiol reagent.

77. (previously added) The method of claim 74, wherein step (b) further comprises: purifying the fusion protein from the extracellular preparation.

78. (previously added) The method of claim 74, wherein the step of purifying the fusion protein further comprises binding to a chitin resin column.

79. (currently amended) The method of claim 74, wherein the plasmid is capable of expression in ~~at least one cell type~~ a host cell selected from the group consisting of a bacterial, a yeast, a plant, an insect and a mammalian host cell ~~type~~.

80. (currently amended) A method for cyclization of a target protein; comprising:

adding a thiol reagent, to a fusion protein comprising a target protein and (i) an intein or modification thereof at a C-terminus of the target protein and (ii) a cysteine or selenocysteine at an N-terminus of the target protein, ~~a thiol reagent for cleaving~~ in order to induce cleavage of the intein from the

target protein ~~so as to form~~ and the formation of a C-terminal thioester on the target protein; and

permitting intramolecular ligation of the C-terminal thioester to the N-terminal cysteine or selenocysteine for cyclization of the target protein.

81. (cancelled)

82. (currently amended) A method for forming a polymer ~~polymerizing a plurality of~~ by intermolecular ligation between target proteins ~~of one type~~ in a preparation, ~~said~~ the method comprising the steps of:

(a) forming each target protein having a C-terminal thioester and an N-terminal cysteine or selenocysteine by cleaving a first and second intein or modifications thereof from a fusion protein, the fusion protein comprising a target protein fused to the first intein at the C-terminal end and the second intein at the N-terminal end ; and

(b) allowing intermolecular ligation between target proteins by reacting the C-terminal thioester of one target protein with the N-terminal cysteine or selenocysteine at the N-terminus of another target protein to form a polymer.

~~(a) expressing fusion proteins from a plurality of plasmids in an *in vivo* expression system, a plurality of fusion proteins, the fusion proteins each comprising a target protein having a C-terminus and an N-terminus, the target protein having a first intein or modification thereof fused to the C-terminus, and a second intein or modification thereof fused to the N-terminus, wherein the first intein or modification thereof is capable of being cleaved to produce a C-terminal thioester; and the second intein or~~

~~modification thereof is capable of being cleaved to form an N-terminal cysteine or selenocysteine;~~

~~(b) obtaining an extracellular preparation of the plurality of expressed fusion proteins;~~

~~(c) adding a thiol reagent to the target protein for cleaving the first intein or modification thereof to produce the C-terminal thioester and inducing cleavage of the second intein or modification thereof to produce the N-terminal cysteine or selenocysteine; and~~

~~(d) permitting intermolecular ligation between the C-terminal thioester on one target protein with the N-terminal cysteine or selenocysteine on a second target protein for forming a polymer from a plurality of target proteins.~~

83. (previously added) The method of claim 82, wherein the first intein is an Mth RIR1 intein (SEQ ID NO:24) wherein the Mth RIR1 is modified.

84. (previously added) The method of claim 82, wherein the second intein is an Mth RIR1 intein (SEQ ID NO:24), wherein the Mth RIR1 intein is modified.

85. (previously added) The method of claim 83 or 84, wherein the modification of the Mth RIR1 intein comprises Ala instead of Asn^{134} at the C-terminus or Ala or Ser instead of Cys^{1} at the N-terminus.

86. (previously added) The method of claim 82, wherein the second intein is cleaved from the target protein by modulating temperature, pH, salt or chaotropic agents or combinations thereof.

87. (currently amended) The method of claim 82, wherein the fusion protein is expressed by a plasmid which is capable of expression in ~~at least one cell type~~ a host cell selected from the group consisting of a bacterial, a yeast, a plant, an insect and a mammalian host cell ~~type~~.

88. (cancelled)

89. (cancelled)

90. (currently amended) A modified Mth RIRI intein, the ~~Mth RIRI~~ intein ~~comprise~~ comprising SEQ ID. No. 24 and the modification comprising Ala^{134} at the C-terminus or Ala or Ser instead of Cys^{1} at the N-terminus of the intein.